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ab219046
Human Fibronectin SimpleStep ELISA® Kit

For the quantitative measurement of Fibronectin in human serum, plasma, milk, cell culture supernatant, and cell and tissue extract samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Fibronectin *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Fibronectin protein in human serum, plasma, milk, cell culture supernatant, and cell and tissue extract samples.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Fibronectin is a large glycoprotein present in the extracellular matrix and circulating plasma. Fibronectin is important in many cell adhesion and migration related processes, including wound healing, embryogenesis and nerve regeneration. Differential expression of fibronectin is seen in coronary heart disease, glomerulopathy and tumor cell metastasis. The protein contains binding sites for collagen, heparin and fibrin and is a specific ligand for several integrin adhesion receptors. Fibronectin exists as a dimer or multimer.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Substrate to each well and incubate for 10 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Human Fibronectin Capture Antibody 10X	600 µL	+4°C
Human Fibronectin Detector Antibody 10X	600 µL	+4°C
Human Fibronectin Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BR	6 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
TMB Substrate	12 mL	+4°C
Stop Solution	12 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Anti-tag coated microplate (12 x 8 well strips)	96 Wells	+4°C
Plate Seal	1	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The provided Antibody Diluents and Sample Diluents contain protease inhibitor aprotinin. Additional protease inhibitors can be added if required.
- The provided Cell Extraction Buffer 5X contains phosphatase inhibitors and protease inhibitor aprotinin. Additional protease inhibitors can be added if required.
- The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only):

Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.3 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BR. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BR. Mix thoroughly and gently.

10. Standard Preparation

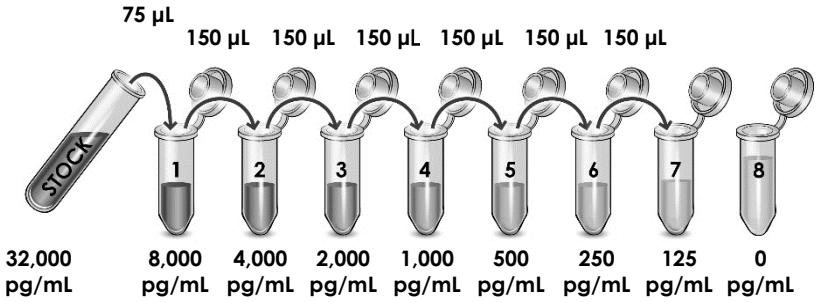
- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 For **serum, plasma, milk, and cell culture supernatant samples measurements**, reconstitute the human Fibronectin protein standard by adding 500 µL of Sample Diluent NS.
For **cell and tissue extract samples measurements**, reconstitute the human Fibronectin protein standard by adding 500 µL of 1X Cell Extraction Buffer PTR.
Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 32,000 pg/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 225 µL of appropriate diluent (see step 10.1) into tube number 1 and 150 µL of appropriate diluent into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1/320,000 – 1/20,000
Plasma - Citrate	1/640,000 – 1/20,000
Plasma - EDTA	1/640,000 – 1/40,000
De-fatted Human Breast Milk	1/800 – 1/100
HepG2 Cell Culture Supernatant	1/32000 – 1/2000
HepG2 Cell Extract	1.56 – 25 µg/mL
SW 480 Cell Extract	3.125 – 100 µg/mL
U87-MG Cell Extract	0.3125 – 5 µg/mL
Liver Tissue Extract	6.25 – 100 µg/mL
Heart Tissue Extract	3.125 – 100 µg/mL
Skeletal Muscle Tissue Extract	3.125 – 100 µg/mL
Colon Tissue Extract	0.63 – 5 µg/mL
Placenta Tissue Extract	0.16 – 1.25 µg/mL

11.1 Plasma:

Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Dilute plasma (citrate) samples at least 1/20,000 into Sample Diluent NS and assay. Dilute plasma (EDTA) samples at least 1/40,000 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1/20,000 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Δ **Note**: Due to the high dilutions required for serum and plasma samples, we recommend to pre-dilute the samples sequentially. As an example the table below demonstrates the steps suggested to generate a final sample dilution of 1:40,000.

Tube #	Sample to Dilute	Volume to Dilute (µL)	Volume of Sample Diluent NS (µL)	Starting Dilution	Final Dilution
1	Neat serum/ plasma	5	245	Neat	1:50
2	Tube #1	5	195	1:50	1:2,000
3	Tube #2	7.5	142.5	1:2,000	1:40,000

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and dilute samples at least 1/2,000 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Milk:

De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute the de-fatted milk samples at least 1/100 in Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

11.5 Preparation of extracts from cell pellets:

11.5.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.5.2 Rinse cells twice with PBS.

11.5.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.5.4 Incubate on ice for 20 minutes.

11.5.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.5.6 Transfer the supernatants into clean tubes and discard the pellets.

11.5.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.5.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.6 Preparation of extracts from adherent cells by direct lysis (alternative protocol):

11.6.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.6.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.6.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.6.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.6.5 Transfer the supernatants into clean tubes and discard the pellets.

11.6.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.6.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.7 Preparation of extracts from tissue homogenates:

11.7.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.7.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.7.3 Incubate on ice for 20 minutes.
11.7.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.
11.7.5 Transfer the supernatants into clean tubes and discard the pellets.
11.7.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.
11.7.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Substrate to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 15 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Δ **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.10 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

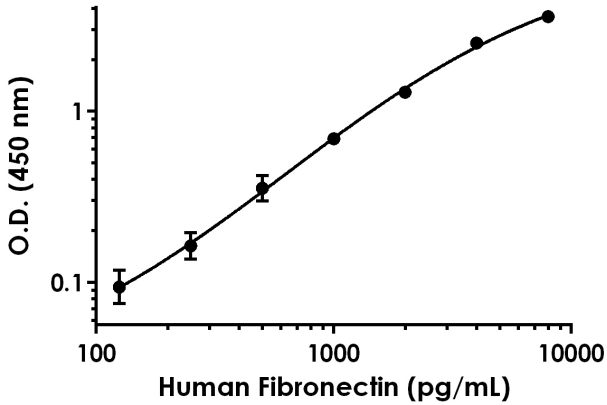
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

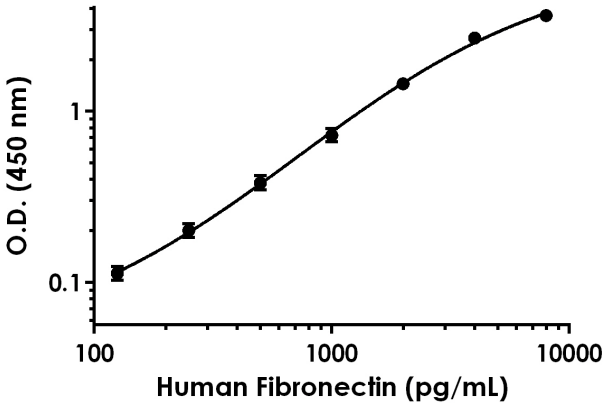
15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.148	0.161	0.154
125	0.234	0.264	0.249
250	0.298	0.340	0.319
500	0.468	0.555	0.511
1,000	0.816	0.874	0.845
2,000	1.453	1.443	1.448
4,000	2.622	2.704	2.663
8,000	3.687	3.760	3.724

Figure 1. Example of human Fibronectin standard curve in Sample Diluent NS. The Fibronectin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.157	0.153	0.155
125	0.275	0.260	0.267
250	0.369	0.342	0.356
500	0.563	0.510	0.537
1,000	0.926	0.833	0.880
2,000	1.668	1.537	1.603
4,000	2.996	2.686	2.841
8,000	3.821	3.733	3.777

Figure 2. Example of human Fibronectin standard curve in 1X Cell Extraction Buffer PTR. The Fibronectin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	16	20.6 pg/mL
1X Cell Extraction Buffer PTR	32	22.3 pg/mL

RECOVERY –

Three concentrations of plasma derived Fibronectin protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1/166,700 Human Serum	93	87 - 102
1/200,000 Human Plasma – Citrate	99	87 - 120
1/200,000 Human Plasma – EDTA	86	81 - 94
1/500 Human De-fatted Breast Milk	102	91 - 111
1/10,000 HepG2 Cell Culture Supernatant (4 days)	102	90 - 117
3 µg/mL HepG2 Cell Extract	89	83 - 100
20 µg/mL SW 480 Cell Extract	90	83 - 100
2.5 µg/mL U87-MG Cell Extract	92	80 - 105
10 µg/mL Human Liver Tissue Extract	117	99 - 131
30 µg/mL Human Heart Tissue Extract	97	81 - 118
30 µg/mL Human Skeletal Muscle Tissue Extract	106	91 - 122
2 µg/mL Human Colon Tissue Extract	99	89 - 112
1 µg/mL Placenta Tissue Extract	90	80 - 104

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Fibronectin was measured in serum, plasma, de-fatted breast milk, and cell culture supernatant (SN) samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	1/20,000 Human Serum	1/20,000 Human Plasma (Citrate)	1/40,000 Human Plasma (EDTA)	1/100 De-fatted Breast Milk	1/2,000 HepG2 SN (4 days)
Undiluted	pg/mL	6832	6984	5890	5398	5408
	% Expected value	100	100	100	100	100
2	pg/mL	3349	3836	2901	2520	2289
	% Expected value	98	110	99	93	85
4	pg/mL	1459	1700	1335	1144	1174
	% Expected value	85	97	91	85	87
8	pg/mL	765.2	838.8	733.9	638.1	616.5
	% Expected value	90	96	100	95	91%
16	pg/mL	383.3	450.1	381.8	NL	287.1
	% Expected value	90	103	104	NL	85

NL – Non-Linear

Native Fibronectin was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	25 µg/mL HepG2 Cell Extract	100 µg/mL SW 480 Cell Extract	5 µg/mL U87-MG Cell Extract
Undiluted	pg/mL	6602	6291	1860
	% Expected value	100	100	100
2	pg/mL	3423	3508	909.3
	% Expected value	104	112	98
4	pg/mL	1735	1587	428.9
	% Expected value	105	101	92
8	pg/mL	913.2	767.8	213.6
	% Expected value	111	98	92
16	pg/mL	483.2	360.7	102.7
	% Expected value	117	92	88

Native Fibronectin was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

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Dilution Factor	Interpolated value	100 µg/mL Liver Tissue Extract	100 µg/mL Heart Tissue Extract	100 µg/mL Skeletal Muscle Tissue Extract	5 µg/mL Colon Tissue Extract	1.25 µg/mL Placenta Tissue Extract
Undiluted	pg/mL	7282	4817	4758	2973	1405
	% Expected value	100	100	100	100	100
2	pg/mL	4040	2273	2109	1481	607.0
	% Expected value	111	94	89	100	86
4	pg/mL	1801	1199	1020	608.6	281.5
	% Expected value	99	100	86	82	80
8	pg/mL	929.9	662.7	499.6	295.8	143.8
	% Expected value	102	110	84	80	82
16	pg/mL	510.3	318.3	270.9	NL	NL
	% Expected value	112	106	91	NL	NL

NL – Non-Linear

PRECISION –

Mean coefficient of variations of interpolated values from three concentrations of serum within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	3	5
CV(%)	5.0	8.3

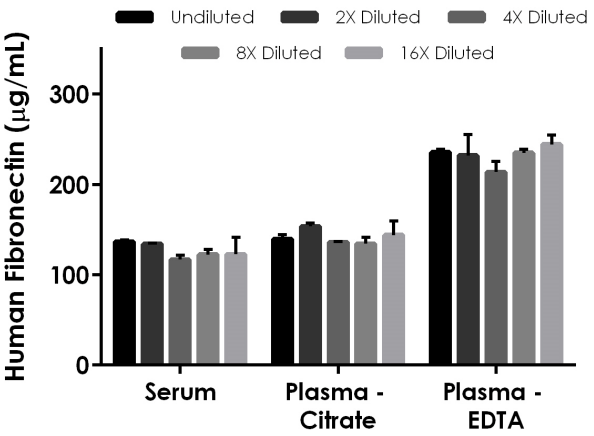

Figure 3. Interpolated concentrations of native Fibronectin in human serum and plasma samples. The concentrations of Fibronectin were measured in duplicates, interpolated from the Fibronectin standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 1/20,000, plasma (citrate) 1/20,000, and plasma (EDTA) 1/40,000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 126.5 µg/mL in serum, 141.5 µg/mL in plasma (citrate), and 232.1 µg/mL in plasma (EDTA).

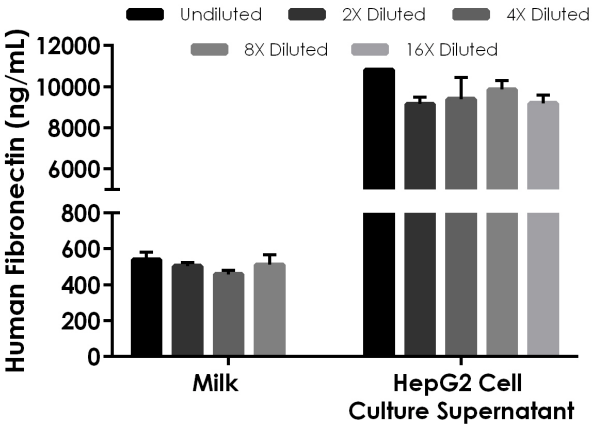

Figure 4. Interpolated concentrations of native Fibronectin in human breast milk and HepG2 cell culture supernatant (4 days) samples. The concentrations of Fibronectin were measured in duplicates, interpolated from the Fibronectin standard curves and corrected for sample dilution. Undiluted samples are as follows: milk 1/100, and HepG2 cell culture supernatant 1/2000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 502.9 ng/mL in milk, and 9683 ng/mL in HepG2 cell culture supernatant.

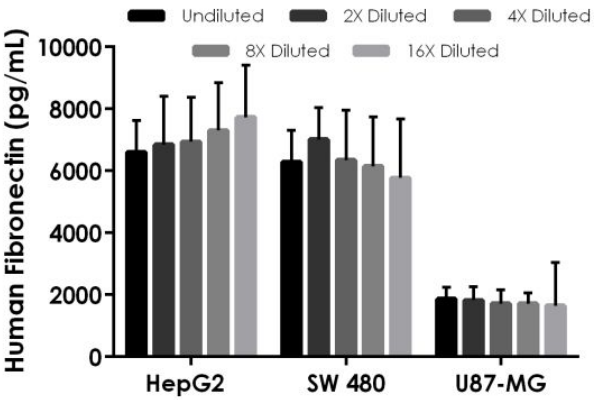

Figure 5. Interpolated concentrations of native Fibronectin in human HepG2 cell extract samples based on a 25 µg/mL extract load, SW 480 cell extract samples based on a 100 µg/mL extract load, and U87-MG cell extract samples based on a 5 µg/mL extract load. The concentrations of Fibronectin were measured in duplicate and interpolated from the Fibronectin standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 7085 pg/mL in HepG2 cell extract, 6314 pg/mL in SW 480 cell extract, and 1749 pg/mL in U87-MG cell extract.

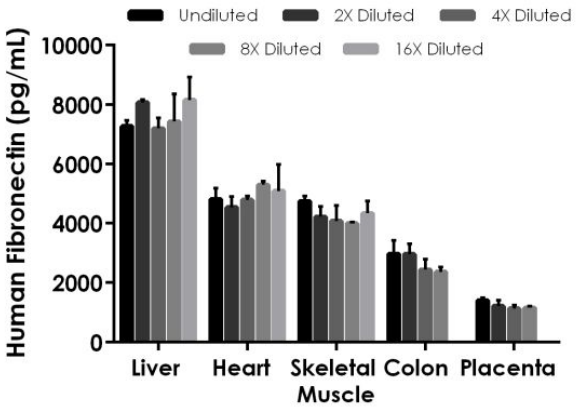

Figure 6. Interpolated concentrations of native Fibronectin in human liver tissue extract based on a 100 µg/mL extract load, heart tissue extract based on a 100 µg/mL extract load, skeletal muscle based on a 100 µg/mL extract load, colon tissue extract based on a 5 µg/mL extract load, and placenta tissue extract based on a 1.25 µg/mL extract load. The concentrations of Fibronectin were measured in duplicate and interpolated from the Fibronectin standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 7634 pg/mL in liver tissue extract, 4910 pg/mL in heart tissue extract, 4278 pg/mL in skeletal muscle tissue extract, 2684 pg/mL in colon tissue extract, and 1224 pg/mL in placenta tissue extract.

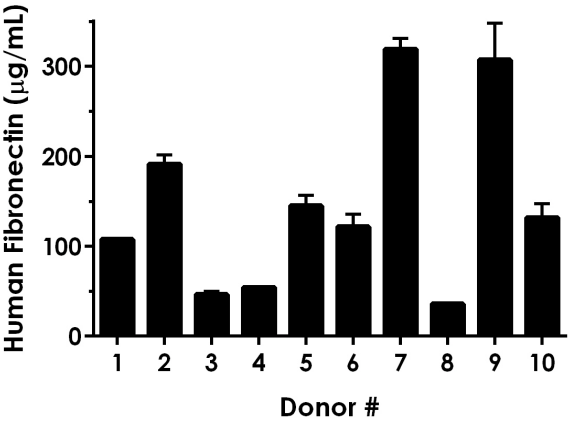

Figure 7. Serum from ten individual healthy male human donors was measured in duplicate. Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 146.2 µg/mL with a range of 35.99 – 319.2 µg/mL.

17. Assay Specificity

This kit recognizes both native and recombinant human Fibronectin protein in serum, plasma (citrate and EDTA), milk, cell culture supernatant, and cell and tissue extract samples only.

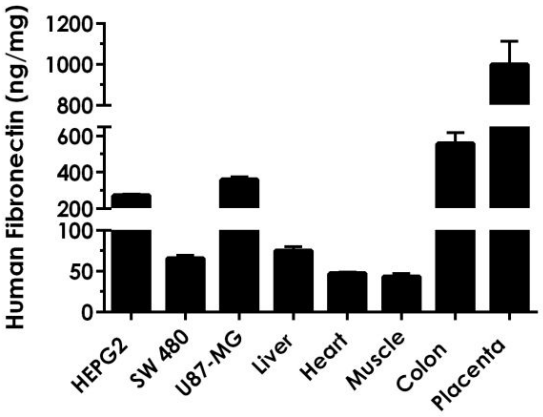

Figure 8. Interpolated concentrations of native Fibronectin in human cell and tissue extract samples. The concentrations of Fibronectin were measured in three different dilutions in duplicate and interpolated from the Fibronectin standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted in ng of Fibronectin per mg of extract (mean +/- SD, n=3). Fibronectin concentration was determined to be 271.8 ng/mg in HepG2 cell extract, 65.52 ng/mg in SW 480 cell extract, 359.6 ng/mg in U87-MG cell extract, 75.22 ng/mg in liver tissue extract, 47.20 ng/mg in heart tissue extract, 43.52 ng/mg in skeletal muscle tissue extract, 557.9 ng/mg in colon tissue extract, and 998.8 ng/mg in placenta tissue extract samples.

Plasma heparin, saliva, and urine samples are not compatible with this kit.

CROSS REACTIVITY

Recombinant human Anastelin was prepared within the working range of the assay and assayed for cross reactivity. No cross-reactivity was observed.

18. Species Reactivity

This kit recognizes human Fibronectin protein.

Other species reactivity was determined by measuring 1/100,000 serum (mouse, rat, cow) or plasma (Rhesus macaque) samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum or plasma assayed at the same dilution.

Species	% Cross-reactivity
Mouse (serum)	98
Rhesus macaque (plasma)	111

Reactivity < 3% was determined for the following species:

- Rat
- Cow

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

20. Notes

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